is considerably increased when highly cross-linked polymer sorbents or chemically modified polymers are used.

The phenolics of environmental concern can be determined at levels required by legislation by using online SPE-RPLC and different sorbents can be used, although the best ones are the highly cross-linked polymeric sorbents.

Figure 3 shows the chromatograms of 10 mL of river water sample obtained by online trace enrichment with a styrene-divinylbenzene (PLRPs) precolumn and HPLC with electrochemical and UV detectors. The figure also shows the chromatograms of the same sample spiked at 1 μg L^{-1} of each phenol.

As regards other kinds of sample, online SPE-RPLC has been successfully applied to the determination of phenolic compounds in beverages, urine and so on.

Figure 4 shows the chromatograms of a sherry, obtained after direct injection of 20 μL (A) and after online SPE of 5 mL of sample (B) and HPLC with UV detection at 280 nm.

In summary, liquid chromatography is a suitable technique for determining phenolic compounds in different kinds of samples. Different detection techniques may be used, some of which, such as EC, enable low levels of phenols to be detected. Mass spectrometry, mainly with APCI, is good for confirming the presence of a phenol in samples at low levels. SPE is the sample-handling technique which is most used for both preconcentration and clean-up of the samples. The combination of this technique, in both the off- and online mode, enables phenols to be detected at low μg L^{-1}.

See also: **II/Chromatography: Liquid:** Derivatization; Detectors: Mass Spectrometry; Detectors: Ultraviolet and Visible Detectors. **Extraction: Solid-Phase Extraction. III / Phenols:** Gas Chromatography; Solid-Phase Extraction.

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DropBooks

Solid-Phase Extraction

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Introduction

Nowadays, sample preparation plays the major role in analysis, as the increasing complexity of samples frequently makes direct analysis impossible. With particular samples, this act is often the limiting factor in the analysis. The aims of sample preparation are as follows: to put the sample in the appropriate physical state for analysis; to clean up the analytes (separate the interference from analytes), and enrichment of analytes. There are many methods of sample preparation which lead to correct analytical results. Features and applications of these methods are presented in numerous compilations and monographs. In this

article, only techniques in which solid-phase extraction (SPE) or solid-phase microextraction (SPME) are applied to samples containing phenols are presented.

Phenols form the group of aromatic compounds with one or more hydroxyl groups. These chemicals are produced as waste in oil refineries, coke plants and some chemical manufacturing, such as the pulp and paper, antioxidant, plastics and dye industries. They also occur in the environment as biodegradation products of humic substances, tannins and lignin. Nitro- and chlorophenols are the main degradation products of many organophosphorous insecticides and chlorinated phenoxyacid herbicides.

The need of analyses focused on the content of phenol results from the fact that phenols, especially chlorophenols, are toxic at concentration levels of a few $\mu g\,L^{-1}$. Even at very low concentrations, phenols affect the taste and odour of fishes and drinking water. A number of phenol compounds are listed (e.g. by the European Community, US Environmental Protection Agency (EPA)) as priority pollutants. Recent regulations enacted in many European countries state that phenols cannot be present in water destined for human consumption at individual levels exceeding $0.1\,\mu g\,L^{-1}$. This means that analytical methods capable of detecting phenols at tens of $ng\,L^{-1}$ are necessary. These methods should also be able to detect phenols at $1–3\,\mu g\,L^{-1}$ levels in surface water samples, because several phenols are toxic to aquatic life. There are various methods to determine phenols in samples. Most important are:

- Spectrophotometric methods based on the reaction of 4-aminoantipyrine with phenols (in this method only total phenols can be determined);
- Gas chromatography with derivatization and electron-capture detection (standard EPA method);
- Liquid chromatography with UV, amperometry, mass spectrometry, fluorescence or chemiluminescence (after derivatization) detection;
- Thin-layer chromatography (usually used as a screening method).

Unfortunately, each of the above analytical procedures is inadequate for monitoring traces of phenols in complex matrices. Each requires enrichment and clean-up techniques. In many laboratories, the procedures for phenol trace enrichment are carried out by means of distillation or liquid–liquid extraction. The disadvantages of these methods, such as enrichment of contaminants, time, emulsion formation, and the use of relatively large amount of solvents, are well known. Consequently, SPE and SPME were established as promising alternative sample preparation techniques.

Specificity SPE of Phenols

As a first approach, SPE can be described as a simple chromatographic process with aqueous media as the mobile phase and the sorbent as the stationary phase. During the enrichment step, the analytes are retained by the sorbent and not eluted by water. Advantages of the method are low cost, minimal consumption of organic solvent and convenience. The techniques can be incorporated easily into fully online set-ups, facilitating automation. Various solid phases with different selectivity, such as octadecyl- or phenyl-bonded silicas, ion exchange materials, polymer resins, porous graphitic carbon, and graphitized carbon black, are used for extraction with varying results. The specificity of extracting phenols by SPE must first consider all the following parameters:

- Phenol and substituted-phenols are acids. Extraction depends on the degree of ionization and can be controlled by the sample pH. Adjusting the pH of the solution or solvent to be used for elution of about 2 units away from the pK_a affords the possibility of achieving quantitative retention and elution of the analyte. For instance, adjustment of the pH of the solution containing 2,4-dinitrophenol (pK_a 4.09) to two pH units above the pK_a of analyte results in approximately 99% ionization. The analyte is deprotonated and forms the conjugate base, which can be retained by anion exchange sorbents. Consequently, the pH of the elution solvent should be 2 pH units below pK_a values. At this pH the analytes are no longer negatively charged and can be eluted from the sorbent. Similarly, at pH = 7, samples containing, e.g. phenol (pK_a 9.89) or 2,4-dimethylphenol (pK_a 10.63), acid–base equilibrium of these substances shifts significantly toward the neutral form, which is readily retained on nonpolar phases.
- Phenols, like polar analytes, are slightly volatile and can be partially degraded when heated. Online SPE procedures are often more convenient to analyse these substances. Online coupling of SPE to liquid or gas chromatography of phenols is easily performed in many laboratories with the automated devices commercially available. In these systems, the extraction pre-column is placed in the sample-loop position of a six-port switching valve. After conditioning, sample application and clean-

ing, the pre-column is coupled to an analytical column by switching the valve to the inject position. Phenols are then eluted directly from the SPE pre-column by a suitable mobile phase, which also permits their chromatographic separation. Advantages of online SPE are that there is no risk of contamination or loss of analyte as there is no sample manipulation between preconcentration and analysis; quantitative results are expected; and the entire sample is transferred and analysed, allowing small sample volume to be used.

- The characteristic matrix for phenols is water. The presence of relatively large amounts of humic substances in aqueous samples can make SPE techniques less effective in extracting phenols. Formation of chemical complexes with fulvic acids and saturation of adsorbent sorption sites by these acids (which are rather scarcely retained by adsorbents) can lead to considerable analytes losses.
- The need for derivatization of phenolic samples is motivated by improved sensitivity and gas chromatographic performance. Derivatization with acetic anhydride to form phenol acetates is one of the simplest and most practical methods. This method does not require extraction of the phenols to organic solvent prior to the addition of the derivatizing reagent.

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SPE of Phenols by Reversed Phases Based on Silica Gel

Synthetic silica with a nominal pore diameter of 6 nm and a particle size distribution of 40–63 μm is used for the synthesis of the chemical-bonded stationary phases. A broad range of phases for selective sample preparation are available. This range covers nonpolar, medium polar and strongly polar phases (ion exchangers).

Work concerning applications of reversed-phase SPE (RP-SPE: using octadecyl- or phenyl-bonded silicas as adsorbents) for extraction of phenols from water have lost practical utility. Additionally, if the matrix contains lipophilic compounds, selective isolation of such phenols using reversed phases based on silica gel is difficult. In these cases, isolation using an anion exchange mechanism is more selective.

From a number of publications concerning the efficiency of different phases for the isolation of phenols, two (both by Puig and Barceló) demand more attention. The first concerns the application of reversed phases based on silica gel in offline and online systems. Offline experiments were performed using C_{18} extraction discs and, for comparison purposes, styrene-divinylbenzene (SDB) discs. After disc activation with 10 mL of acetone and 10 mL methanol, 1 L of groundwater spiked with phenol at 5 $\mu g\,L^{-1}$ and acidified to pH 2 was passed through the discs. Analytes were eluted using three aliquots of methanol of 20 mL total volume. Eluates were concentrated to 1 mL and analysed by liquid chromatography (LC-UV) and liquid chromatography–mass spectrometry (LC-MS) methods. Online SPE-LC was carried out on stainless steel pre-columns (10×2 mm). Extraction efficiency was determined for the following phases, based on silica gel: C_{18}, C_{18}-OH, C_8, phenyl, cyclohexyl and, for comparison purposes, CN and polystyrene divinylbenzene sorbent (PLRP-S). Pre-columns were conditioned with 10 mL methanol and 5 mL water (pH 3). After extraction of spiked samples, acidified to pH 2, and washing the sorbent with 2 mL of water, phenols were eluted by passing the mobile phase (mixture of water and methanol–acetonitrile 1 : 3 containing 1% acetic acid) directly through the analytical column. The authors evaluated recoveries (**Table 1**), breakthrough volume, matrix effects and the influence of extraction column dimensions on band broadening. The size of pre-column is an important parameter in the coupling methods because the profile of concentrated analytes transferred to the analytical column should ideally be as narrow as possible at the beginning of the separation. It was shown that the more non-polar sorbents (C_{18} and C_8) gave good results for the more nonpolar phenols (e.g. trichlorophenol and pentachlorophenol). With these phases the concentrated volume, prior to breakthrough of the other target compounds, was less than 10 mL. The results concerning the remaining phenols were only slightly improved by using monofunctional C_{18}-OH.

SPE of Phenols on Polymeric Phases

At the beginning of the 1970s, a new nonionic styrene-divinylbenzene copolymer, with a particle size distribution appropriate for sample preparation (40–120 μm), was introduced by Junk *et al*. Due to its very large surface area (approximately 1200 $m^2\,g^{-1}$), its adsorption capacity for organic compounds is excellent. That adsorbent contains a relatively large number of active aromatic sites capable of interactions with the aromatic phenols, which considerably improves the enrichment of these analytes. The other advantage of the styrene-divinylbenzene copolymer is excellent chemical and mechanical stability. As previously mentioned, Puig and Barceló performed a comparative study of the performance of four sorbents for online SPE followed by LC of phenol

Table 1 Mean percentage recoveries and standard coefficients of variation (CV) of phenols in different sorbents in ground water when working with online procedures using a 10 × 2 mm i.d. stainless-steel pre-column

Compound	*Sorbent*						
	C_{18}	C_{18}/OH		Cyclohexyl		PLRP-S	
Concentration ($\mu g\ L^{-1}$)	*5*	*0.5*	*5*	*0.5*	*5*	*0.5*	*5*
Catechol	< 20	< 20	< 20	< 20	< 20	< 20	< 20
Phenol	< 20	< 20	< 20	< 20	27 ± 8	< 20	37 ± 6
4-Methylphenol	25 ± 6	42 ± 7	54 ± 6	59 ± 5	68 ± 6	69 ± 5	88 ± 6
2,4-Dimethylphenol	57 ± 5	61 ± 3	75 ± 4	67 ± 4	77 ± 5	81 ± 3	96 ± 4
2-Nitrophenol	31 ± 8	50 ± 5	63 ± 4	46 ± 6	61 ± 4	76 ± 4	95 ± 5
4-Nitrophenol	33 ± 7	44 ± 6	56 ± 6	42 ± 5	59 ± 6	78 ± 5	89 ± 7
2,4-Dinitrophenol	30 ± 6	40 ± 5	53 ± 5	40 ± 6	53 ± 5	100 ± 4	99 ± 4
2-Amino-4-chlorophenol	< 20	< 20	< 20	< 20	< 20	< 20	< 20
4-Chloro-3-methylphenol	52 ± 5	58 ± 4	76 ± 3	55 ± 5	70 ± 4	85 ± 6	94 ± 5
2-Chlorophenol	< 20	49 ± 5	52 ± 4	66 ± 4	78 ± 5	76 ± 4	85 ± 4
3-Chlorophenol	< 20	35 ± 6	48 ± 5	68 ± 5	81 ± 5	78 ± 7	90 ± 6
4-Chlorophenol	< 20	41 ± 5	53 ± 4	65 ± 4	73 ± 5	85 ± 6	93 ± 6
2,4-Dichlorophenol	41 ± 5	73 ± 4	85 ± 5	71 ± 7	85 ± 6	81 ± 5	95 ± 3
2,4,6-Trichlorophenol	62 ± 5	72 ± 4	87 ± 6	92 ± 4	97 ± 4	96 ± 5	98 ± 4
2,3,5-Trichlorophenol	70 ± 4	75 ± 4	81 ± 4	90 ± 4	98 ± 5	94 ± 6	99 ± 5
2,3,4-Trichlorophenol	71 ± 5	74 ± 3	87 ± 5	91 ± 6	101 ± 5	95 ± 7	100 ± 5
3,4,5-Trichlorophenol	75 ± 4	78 ± 5	88 ± 4	95 ± 4	98 ± 5	93 ± 5	102 ± 5
Pentachlorophenol	87 ± 4	89 ± 4	97 ± 3	97 ± 5	99 ± 5	100 ± 5	99 ± 3

Spiking level: 0.5 and 5 $\mu g\ L^{-1}$ ($n = 6$ for each phenolic). Sample volume: 10 and 100 mL for 5 and 0.5 $\mu g\ L^{-1}$ concentration respectively. Reproduced with permission from Puig and Barceló (1995).

compounds in water. Three of these PLRP-S, LiChrolut EN and Isolut ENV) were phases based on styrene-divinylbenzene copolymer; the fourth was porous graphitic carbon (PGC) (**Table 2**), detection limits (to 0.1 $\mu g\ L^{-1}$) and breakthrough volumes. Improved breakthrough values were obtained with LiChrolut EN and Isolut ENV. PGC gave good results only for aminophenols. It was demonstrated that acidification of the sample is necessary to avoid binding of some phenols to humic substances and to prevent their partial deprotonation.

SPE of Phenols on Carbon Phases

The selective retention of phenols contained in water can be realized using PGCs or graphitized carbon blacks (GCBs).

It was demonstrated that retention of some polar compounds in water could be high when using PGC. In one experiment an online technique, coupling SPE and LC, was applied to the analysis of water-soluble organic pollutants, such as aminophenols. (These products are degradation products of aniline and some pesticides and their extraction from water is difficult owing to their high polarity. The authors demonstrated that a PGC pre-column cannot be coupled online with the widely used and efficient C_{18} analytical column, but could be used with a PGC analytical column. Separations on C_{18} is achieved with water-rich mobile phases, which are unable to desorb analytes that are more retained by PGC pre-column. Band broadening, the influence of the matrix, and recoveries were evaluated. For example, retention of 4-aminophenol on PGC, which is not retained on C_{18} and only slightly on PRP-1, was 10 times higher in compared with PRP-1.

The other carbon materials recommended for phenol extraction are GCBs. They are nonporous sorbents with surface areas ranging from 8 to 100 $m^2\ g^{-1}$. The adsorbent contains chemical heterogeneities on its surface. In the presence of water, these surface to form positively charged impurities. Thus GCB behaves both as a nonspecific sorbent and an anion exchanger. This feature is used for rapid and simple isolation of acidic analytes from co-extracted base-neutral species by differential elution. Elution by neutral eluents allows for transfer to solution of base-neutral and very weak acidic compounds. The most acidic compounds (e.g. phenols having pK_a values lower than 7) remain on the adsorbent and may be transferred to the solution by the acidified eluent. Commercially referred to as Carbograph 1 or Car-

Table 2 Mean percentage recoveries ± standard deviation of phenol compounds in groundwater using different sorbent and working with online liquid–solid extraction (LSE) using a 10 × 2 mm i.d. stainless-steel pre-column

Compound	*Sorbent*			
	PLRP-S	*LiChrolut EN*	*Isolute ENV*	*PGC*
Catechol	< 20	55 ± 9	57 ± 8	61 ± 7
Phenol	34 ± 5	67 ± 7	62 ± 7	54 ± 6
4-Methylphenol	69 ± 6	75 ± 6	82 ± 5	52 ± 7
2,4-Dimethylphenol	81 ± 4	98 ± 4	92 ± 4	n.d.
2-Nitrophenol	76 ± 5	88 ± 5	88 ± 5	n.d.
4-Nitrophenol	78 ± 5	84 ± 6	100 ± 4	n.d.
2,4-Dinitrophenol	100 ± 4	102 ± 5	98 ± 4	n.d.
2-Amino-4-chlorophenol	< 20	< 20	< 20	87 ± 6
4-Chloro-3-methylphenol	85 ± 5	92 ± 6	88 ± 5	n.d.
2-Chlorophenol	76 ± 4	86 ± 6	81 ± 4	85 ± 7
3-Chlorophenol	78 ± 6	83 ± 5	79 ± 5	88 ± 6
4-Chlorophenol	85 ± 6	84 ± 5	80 ± 4	88 ± 5
2,4-Dichlorophenol	81 ± 3	94 ± 5	92 ± 3	n.d.
2,4,6-Trichlorophenol	96 ± 4	103 ± 5	99 ± 5	n.d.
2,3,5-Trichlorophenol	94 ± 5	96 ± 4	101 ± 6	n.d.
2,3,4-Trichlorophenol	95 ± 5	101 ± 4	105 ± 6	n.d.
3,4,5-Trichlorophenol	93 ± 5	99 ± 4	98 ± 4	n.d.
Pentachlorophenol	100 ± 4	100 ± 3	99 ± 5	n.d.

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bopack, GCB has proved to be a valuable sorbant for SPE. However, it was demonstrated that Carbograph 4 is more efficient than Carbograph 1 in extracting very polar compounds from large volumes of water (**Table 3**). A robust and selective liquid chromatography method was developed enabling rapid determination of 11 US EPA phenols in samples in the ng L^{-1} range. The method involved extraction of phenols by a reversible 0.5 g Carbograph 4 cartridge and re-extraction by an eluent containing a quaternary ammonium salt. Phenols were directly analysed by LC-UV, after derivatization to appropriate acetyl derivatives. Recovery of phenols was higher than 90%.

SPE of Phenols on Anion Exchangers

For phenol analysis, synthetic anion exchangers (polymers and chemical-bonded silicas) are generally applied because of their durability and chemical resistance. The typical functional groups of strong or weak basic anion exchangers are $-CH_2-N^+(CH)_3$ and $-N^+H(R)_2^-$ respectively.

An application of ion exchangers for selective determination of phenols in water by a two-trap tandem extraction system followed by LC has been proposed. The first extraction column was filled with 300 mg GCB and the second with 50 mg of a strong anion exchanger (SAX, commercially referred to as Sephadex QAE A-25). After the water sample had been passed through the GCB cartridge, the latter was connected to the SAX cartridge. Base-neutral species adsorbed on GCB were removed by a neutral eluent. Co-eluted, very weakly acidic phenols were selectively re-adsorbed on the SAX sorbants. Next, avoiding disconnection of the cartridges, an acidified eluent was allowed to flow through the two cartridges to recover the most acidic phenols from the GCB and the least acidic phenols from the SAX. After partial removal of the solvent, the final extract was analysed by LC-UV. Recoveries of 17 phenols added to 2 L of drinking water were higher than 90%.

Recoveries from 1 L of tap water and seawaters are presented in **Table 4**. In one process it was possible to concentrate both more and less polar phenols. The method is characterized by high selectivity; only acidic organic compounds can interfere with the phenols. Anionic surfactants (frequently occurring in aqueous environmental samples) are not eluted, because the formic acid contained in the second mobile phase is unable to displace the anionic surfactants from positively charged sorption sites populating the GCB surface.

Solid-phase Microextraction of Phenols

SPME can be used to concentrate volatile, semivolatile and nonvolatile phenols in both liquid

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Table 3 Recovery of phenol on extracting 4 L of drinking water by cartridges containing two different GCB sorbent materials

Compound	*Recovery (%)*[a]		
	Carbograph 1	*Carbograph 4*	
	Forward elution	*Forward elution*	*Backflush elution*
Phenol	26[b]	95	96
4-Nitrophenol	93	65	99
2-Chlorophenol	53	97	98
2,4-Dinitrophenol	93	15	96
2-Nitrophenol	97	97	98
2,4-Dimethylphenol	63	98	100
4-Chloro-3-methylphenol	98	96	99
2,4-Dichlorophenol	96	98	98
4,6-Dinitro-2-methylphenol	97	10	95
2,4,6-Trichlorophenol	93	96	98
Pentachlorophenol	100	36	101

[a]Mean values obtained from duplicate measurements.
[b]Recoveries of phenols remained unaltered in the backflushing mode.
Reproduced with permission from Di Corcia *et al.* (1996).

and gaseous samples. This method, in which sorbent-coated silica fibres are used to extract analytes, is superior to other sample preparation methods. The fibre is introduced into the sample or headspace and phenols (or other organic substances) establish equilibrium and partition on to the phase. Next, the analytes are thermally desorbed from the fibre to the gas chromatograph. SPME is a fast, simple and sensitive technique which does not require the use of solvents. The method is significantly simpler than conventional SPE, thereby reducing the potential for loss of analyte during the extraction process. Most of the SPME methods are based on a poly(dimethylsiloxane) coating, which is relatively nonpolar. In the case of phenol extraction, it is necessary to use a more polar phase or a derivatization procedure to reduce their polarity and improve chromatographic properties.

The basis of SPME optimization for the determination of phenols was established by Buchholz and Pawliszyn. The subject of the research was to evaluate the derivatization step by increasing the affinity of the phenols for the poly(dimethylsiloxane) coating, comparison of the affinity of phenols for the poly(dimethylsiloxane) coating and a more selective, polar coating (a poly(acrylate)), the influence of acid and salt on the enhancement of phenols, evaluation of time equilibration on the equilibrium distribution constant between a solid-phase coating and water and an evaluation of the above parameters on carry-over. Headspace analysis of phenols was also evaluated. The most important conclusions are:

- The poly(dimethylsiloxane) coating is not suitable for extracting phenol; some chlorophenols and nitrophenols could not be extracted in large enough amounts to be detected by GC.
- The derivatization procedure created compounds with higher affinity for the poly(dimethylsiloxane) coating; the amount of acetate extracted for most of the target analytes was several times greater than the free form (2-nitrophenol was the only compound for which the amount of derivative extracted was less than the free phenol). It was demonstrated that the *in situ* derivatization can be performed directly in the polymeric coating.
- Phenol acetates have significantly better peak shape than the free phenols.
- Poly(acrylate) coating was successful in extracting both polar and nonpolar compounds; the fibre only slightly favours the polar phenols over its nonpolar analogues.
- Only 15 min is required for all analytes to equilibrate with the poly(dimethylsiloxane) coating; with poly(acrylate)-coated fibres, 40 min was required. That can be explained by the fact that the poly(dimethylsiloxane) phase is a liquid, whereas the poly(acrylate) phase is a solid.
- When the extraction is performed under saturated salt condition, 5.5 times more phenol was extracted compared to the control sample (sample of the same concentration at neutral pH and with no salt added).
- The salting-out effect was observed for high pK_a phenols; this results in a positive increase in the

Table 4 Recovery of phenol on extracting 1 L of tap water and seawater by the proposed method compared with those from two other extraction methods

Compound	*Recovery (%)*[a]					
	Anion exchanger		C_{18}		*This method*	
	Tap	*Sea*	*Tap*	*Sea*	*Tap*	*Sea*
Guaiacol	12	5	3	8	98	93
p-Nitrophenol	96	56	6	9	99	98
p-Cresol	27	7	8	10	97	92
6-Chlorovanillin	94	33	20	28	100	99
o-Chlorophenol	97	14	6	11	98	95
2,4-Dinitrophenol	98	95	7	13	96	98
o-Nitrophenol	93	47	7	9	97	97
2,4-Dimethylphenol	44	14	20	36	98	97
Bromoxynil	97	89	68	84	101	101
2,4-Dichlorophenol	94	73	20	32	99	100
4,6-Dinitro-*o*-cresol	98	93	40	56	95	95
loxynil	102	96	101	98	100	100
2,4,6-Trichlorophenol	98	89	88	85	98	98
3,4,5-Trichlorosyringol	99	90	93	94	96	97
Tetrachlorophenol	100	93	98	98	99	100
Dinoseb	99	94	96	97	101	01
Pentachlorophenol	101	97	99	100	97	97

[a]Mean values obtained from triplicate measurements. Reproduced with permission from Di Corcia *et al.* (1993).

amount extracted for these phenols; for phenols which have a considerable portion of their molecules in the ionized form, the salting-out effect is a negative factor.

- Matrix significantly affects the extraction of phenols, especially nitrophenols and heavier chlorinated phenols. These effects can be overcome by normalization to extreme acid and salt conditions. Headspace is another method of reducing matrix influence.
- If the fibre is directly exposed to samples which are high in particulate matter, material from the matrix could coat the solid phase and interfere with extraction. To avoid this, the headspace above the liquid should be sampled. The success of this method depends on the transfer of analytes from the aqueous phase to the headspace. Most of the target phenols could be enriched in the headspace by decreasing their solubility in the aqueous phase through saturation with sodium chloride and acidification to pH below 1. However, even with these extreme conditions, most phenols needed 1–2 h to reach equilibrium.

Conclusions

The trace determination of phenols in environmental samples requires specific strategies. Analytical methods need to be sensitive selective, rapid and simple. Both SPE and SPME techniques with a variety of sorbents for enrichment and purification are used. In the last decade, several online systems for SPE-LC or SPE-GC have been published. Online SPE procedures provide greater sensitivity, lower sample volumes, lower consumption of organic solvent, higher automation and better reproducibility. SPME is rarely applied and seems to vary with phenol concentration.

See also: **II/Extraction:** Solid-Phase Extraction. **III/Solid Phase Microextraction:** Environmental Applications.

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Thin-Layer (Planar) Chromatography

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Introduction

Phenols have played an important part in thin-layer chromatography (TLC) since its inception as a general separatory method. The phenolic group occurs in a wide variety of natural replenishable organic compounds from the amino acids (tyrosine), the alkaloids (morphine), the steroids (estrone), glycosides (arbutin, carminic acid, flavonols), perfume ingredients (methyl salicylate, eugenol), phenolic lipids (cashew phenols, urushiol) and the tetracyclines to the cannabinoids. Fractions derived from fossil fuels by distillation or by synthesis contain phenol itself, the cresols, xylenols and polynuclear compounds. Many purely synthetic products, notably drugs (aspirin, paracetamol), technical products (*t*-butylphenol, nonylphenol, and other intermediates such as chloro- and nitrophenols extend the range of substances as candidates for TLC.

The classical separations of structural isomers of monohydric, dihydric and polyhydric phenols and their derivatives have generally formed the basis for subsequent applications to mixtures of more complex compounds. All these initial studies were essentially qualitative and have led over the past four decades to quantitative work aimed at the determination of a single phenolic substance in a mixture or of the total phenolic composition.

The original TLC adsorption method with silica gel was soon supplemented by the partition technique by coating the adsorbent with a paraffinic compound or by silanization. These reversed-phase (RP) approaches have, with the introduction of excellent commercial plates, led to the routine use of alkylsilyl-bonded silica gel with C_{18}, C_8 or C_2 silyl groups.

In a similar fashion, with the development of uniform particle size adsorbents high performance TLC (HPTLC), combined techniques such as TLC–ultraviolet (TLC-UV), TLC–mass spectrometry (TLC-MS), (TLC-GLC) and TLC–high performance liquid chromatography (TLC-HPLC) have all been applied to the study of phenolic materials.

A wide variety of adsorbent layers have been investigated for the analysis of phenols, including silica gel, alumina, cellulose and polyamides as well as mixtures in certain cases with other inorganic materials such as calcium hydroxide or impregnated with organic additives. The use of argentation TLC with phenolic lipids is well established but other ions, for example Fe(III) and Cu(II), have found limited application.

Solvents from the whole elutropic range have been employed and frequently basic conditions have been found advantageous by the use of ammonia or by the addition of an organic base, although solvents acidified with formic acid have an application. With the availability of silica gel G_{254}, visualization of phenolic substrates is straightforward, although with non-indicating silica gel, the use of dichlorofluorescein, rhodamine 6G or a wide selection of spray reagents has become common practice and almost completely displaced the use of sulfuric acid and thermal charring. Documentation by means of photocopying, photography or by the use of dyeline paper and an ammoniated atmosphere are all effective methods.

In the following examples, the analytical scale TLC separations of a range of isomeric compounds are tabulated. At the preparative scale increased sample loading leads to a loss of resolution and indeed the loading/resolution factor should be examined in all cases.

Separation of Structural Isomers of Phenols and Phenolic Acids

The R_F values of an extensive range of phenolic compounds of synthetic and natural origin have been given in the literature listed in the Further Reading